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Pharmacokinetics of Dacarbazine in the Regional Perfusion of Extremities With Melanoma

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Background: The pharmacokinetics of dacarbazine (DTIC), which has been shown to be an effective therapeutic agent against metastatic melanoma, has not been extensively studied. However, to improve the clinical use of the drug, more information on the kinetics is required.

Methods: A pharmacokinetic study was undertaken in six patients with melanoma of an extremity who were undergoing hyperthermic isolation perfusion with DTIC in order to understand better its clinical pharmacokinetics. Plasma was sampled from the arterial and venous lines of an extracorporeal pump during the perfusion with the systemic vein and urine sampled postperfusion. Samples were analyzed for DTIC, 2-azahypoxanthine (2-AZA), and aminoimidazole carboxamide (AIC). ^{99m}Tc (Technetium) human serum albumin (HSA) was used in the perfusion circuit to monitor the crossover of the perfusate into the systemic circulation during the procedure. The data were analyzed using a compartmental model of sampled body compartments incorporating the isolated extremity.

Results: High tissue DTIC levels were maintained throughout the perfusion, whereas in the systemic circulation, plasma DTIC concentrations, when observed, were 40–100-fold less than those in the perfusate. Almost 70% of the DTIC administered was not recovered in the perfusate after the washout of the extremity.

Conclusions: High levels of DTIC can be maintained in an extremity (i.e., arm or leg) during perfusion.

INTRODUCTION

Among many chemotherapeutic agents, dacarbazine, 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DTIC) achieved the best activity with a modest tumor response rate in metastatic melanoma [1]. In the past, studies reported the pharmacokinetics of this agent using a specific colorimetric assay [2]. The development of a high pressure liquid chromatography (HPLC) assay allowed more accurate studies in human subjects [3,4]. More recently, the advances in simultaneous determination of DTIC and its metabolites, aminoimidazole carboxamide (AIC) and 2-azahypoxanthine 2-(AZA), by HPLC facilitated more detailed studies of this agent [5].

Previous studies of the pharmacokinetics of DTIC investigated primarily the intravenous route of administra-

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tion [3,6]. However, DTIC has been used intra-arterially and in vascular isolation perfusion of an extremity with recurrent melanoma [7–11]. The lack of detailed pharmacokinetic studies for DTIC related to vascular isolation perfusion in humans prompted us to undertake this study. In the past, Ainger and associates [12] reported preliminary data on the pharmacokinetics of DTIC related to perfusion in a canine model.

Although melphalan has been the most commonly used drug for vascular isolation and perfusion of an extremity with melanoma over the past 30 years, its detailed pharmacokinetic studies have been reported only recently [13,14]. In the last 8 years, we used DTIC for vascular isolation and hyperthermic perfusion of extremities with melanoma in 75 patients. We noted dose-related hemotoxicity despite adequate vascular isolation of the extremity during perfusion in 14 out of the first 40 patients perfused with DTIC [10]. The leakage of the perfusate monitored by ^{99m}Tc human serum albumin (HSA) into the systemic circulation in these patients was $<10\%$. Furthermore, the unbound drug was also washed out of the isolated extremity circulation by normal saline at the end of the perfusion. This discrepancy, i.e., the development of the hemotoxicity despite minimal leakage of the perfusate into the systemic circulation during perfusion and an adequate washout of the drug after perfusion, indicated the need to investigate further the pharmacokinetics of DTIC.

MATERIALS AND METHODS

Of 75 consecutive patients with melanoma of an extremity undergoing vascular isolation and hyperthermic perfusion, six subjects were selected at random for a detailed pharmacokinetic study. Of these, four underwent lower extremity and two upper extremity perfusion. The lower extremity perfusion was done through the external iliac vessels, and the upper extremity perfusion was done through the second portion of the axillary vessels. The operative technique was similar to that described previously [10,15,16]. The isolation of the circulation of the extremity was achieved by clamping the iliac or axillary vessels. The collateral circulation through the skin, subcutaneous tissue, and muscles was occluded by an Esmarch tourniquet at the axilla or groin. The tourniquet was wrapped tightly at the groin or axillary crease and held from slipping down by a Steinmann pin passed through the iliac bone or skin at the shoulder joint respectively.

Extracorporeal Circuit

The extracorporeal perfusion circuit (heart lung machine, HLM) consisted of the venous tubing of 1/4" (6.34 mm) inner diameter (ID) draining the blood by gravity from the patient to an oxygenator (BOS-2; American Bentley, Irvine, California). The arterial line of 1/4" ID from the oxygenator was connected via a low flow modular pump (Travenol; Baxter Health Care Corporation, Deerfield, Illinois) to the artery of the isolated extremity (Fig. 1). An infant arterial filter (EC 1440; Pall Biomedical Products, East Hills, NY) was placed between the pump and the arterial entry site. A cardiotomy reservoir (BCR-3500; American Bentley, Irvine, CA) containing the washout solution of normal saline and Dextran 40 in equal amounts was connected to the arterial line on the negative side of the pumphead to control the rate of washout infusion at the end of the perfusion. A Sarns heater-cooler was used to elevate the perfusate temperature in the oxygenator during the procedure. The length of all the lines was kept to a minimum avoiding the loss of heat and minimizing the exposure of the perfusate to operating room light. However, no additional steps were taken to prevent the exposure of the drug in the HLM from light.

Perfusion, Washout Fluids, and Drug Doses

The extracorporeal pump was primed with 700 ml of balanced electrolyte solution containing 3,000 IU of sodium heparin. All patients received systemic heparin 100 IU/kg prior to the clamping of the vessels. The heparinization was not reversed at the end of the procedure. After the circulation of the extremity was isolated, the arterial and venous cannulae were connected to the primed extracorporeal HLM pump. The extremity was then perfused by the heated perfusate. Two liters per minute of 100% oxygen was used in the oxygenator. To keep the pH of the perfusate at 7.4, sodium bicarbonate, 40 cc, was injected in the perfusion circuit at the beginning of perfusion. The waterbath and arterial line temperatures were raised to 42°C and 40°C, respectively.

When the venous return was balanced with arterial input, DTIC was injected into the perfusate in the oxygenator in three to four equally divided doses at 5-minute intervals. The dose of DTIC for lower extremity studies was 2 g/m^2 and for the upper extremity 1.2 g/m^2. The hyperthermic perfusion with DTIC was done for a 1-hour period. The temperature of the arterial line, extremity skin, and muscle (core) was monitored every 5 minutes. The core temperature of the extremity was raised to $\sim$ 38°C in all patients. At the conclusion of perfusion, the drug was washed out with 800 ml and 1,200 ml of saline for the upper and lower extremities, respectively.

During the perfusion, 1 mCi of ^{99m}Tc labeled HSA was added to the perfusate. Leakage of the perfusate into the systemic circulation was then monitored by a gamma detector placed over the chest. At the end of perfusion, the washout perfusate was sampled to detect the recovery of the injected ^{99m}Tc HSA.

The perfusionist monitored and recorded the volume

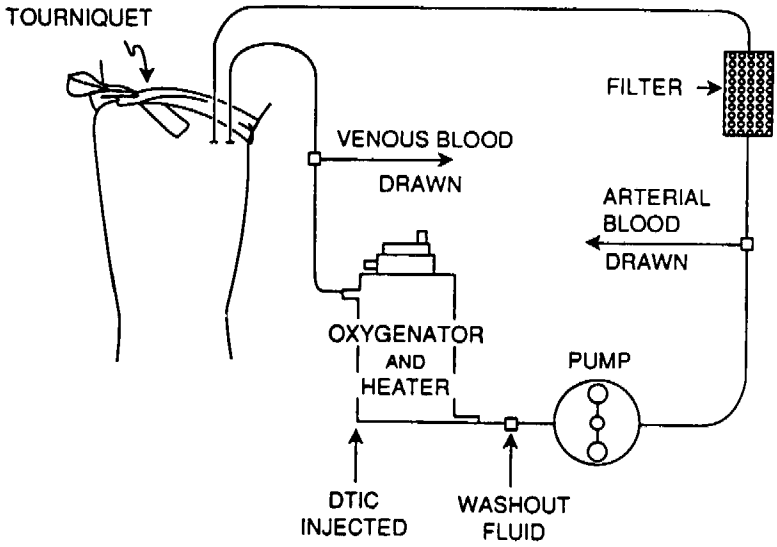

Fig. 1. Labeled schematic representation of the perfusion of patient's extremity.

changes in oxygenator, flow rates, and washout solution throughout the procedure.

Sample Collection

The samples of perfusate, 5 ml each, were simultaneously drawn from the arterial and venous lines of the extracorporeal circuit into heparinized tubes covered with foil to avoid further degradation via light. The plasma was immediately separated by centrifugation and frozen at −70°C.

Samples representing systemic vein blood were drawn from a peripheral vein. Baseline samples from the systemic vein and the perfusion circuit were collected prior to injection of DTIC. During dosing samples were collected 1 minute before the next bolus dose at 4 minutes and 14 minutes, with five additional samples being collected at 20, 30, 40, 50, and 60 minutes from the arterial and venous lines of the perfusion circuit. The blood samples from the peripheral vein were collected every 30 minutes during perfusion and approximately every 2 hours after perfusion for 8 hours and later every 4 hours for 24 hours. A urine sample was collected at the end of the perfusion and then every 2–4 hours after perfusion for 24 hours with the volume recorded and an aliquot frozen for analysis.

Analysis of DTIC, 2-AZA, and AIC

DTIC, 2-AZA, and AIC in plasma and urine were assayed by a gradient elution HPLC procedure that was previously reported [5]. The physiological values for plasma and muscle volumes as well as post perfusion plasma flow rates obtained from the literature [17] are presented in Table I.

Development of Model for In Vitro Binding

A compartmental model for the kinetics of DTIC in the drug delivery system was developed based upon information obtained from studies in the extracorporeal perfusion circuit in the absence of a human subject. The in vitro model (Fig. 2) was based on the assumption that the 25% of drug not recovered at the conclusion of the in vitro study was sequestered by the major components of the circuit (i.e., heart-lung machine (HLM) and/or degraded by light to 2-azahypozanthine (2-AZA), DTIC's photolytic degradation product. Rate constants from compartments that had measured flows in the HLM and volumes were determined as: K = flow from compartment/volume of compartment.

The in vitro model was constructed as separate but parallel components consisting of: (1) measured vol-

TABLE I. Physiological and Perfusion Parameters for the Six Melanoma Patients Who Had Either a Leg or Arm Perfused with Dacarbazine (DTIC)

Patient	1	2	3	4	5	6
Dose (millimoles)	8.8	19.7	17.6	18.7	9.9	20.9
Body weight (kg)	58	65	59	59	78	95
Muscle volume (ml)[a]	385	800	800	800	400	1,100

[a] Values from ref. 17.

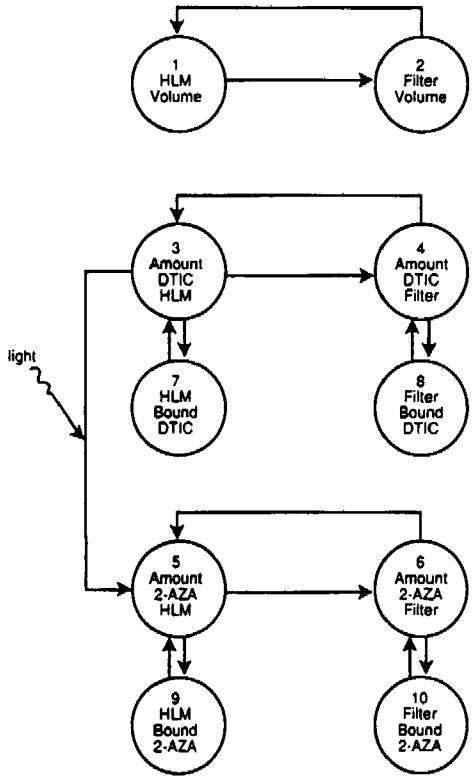

Fig. 2. In vitro model for DTIC based upon the perfusion circuit in Figure 1. The model represents the amount of DTIC and formed 2-AZA within the heart lung machine (HLM), compartments 1 and 3, and arterial filter, compartments 4 and 6, respectively. The volume of perfusion fluid in the HLM and arterial filter is given in compartments 1 and 2 with nonlinear equilibrium binding of both compounds to the HLM and filter respectively (i.e., compartment 7 and 8 for DTIC and compartments 9 and 10 for 2-AZA).

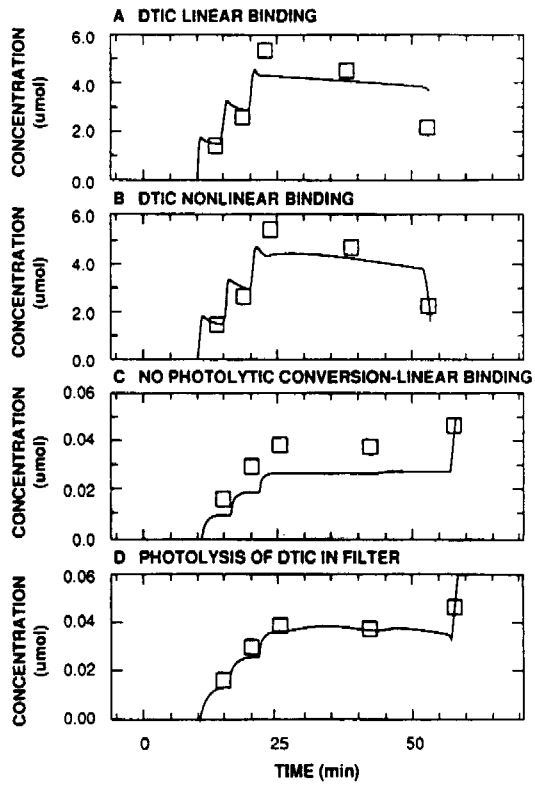

Fig. 3. Effect of linear and nonlinear equilibrium binding to the heart-lung machine and filter on the in vitro fitted levels of DTIC (**A,B**) in the in vitro model. For 2-AZA (**C,D**), the effect of photolytic conversion of DTIC and linear binding is shown using the in vitro model.

umes—compartments 1 and 2 representing HLM and arterial filter, (2) Amount of DTIC within the system (HLM and filter) following multiple bolus administration within HLM, compartments 3 and 4, respectively, (3) 2-AZA formed from DTIC via photolysis—compartments 5 and 6; the HLM and tubing was not protected from light during the procedure, and arterial filter and HLM as potential binding surfaces, compartments 7–10, to account for the 25% of DTIC not recovered during in vitro perfusion.

The volumes reflected the initial volume within the HLM, contiguous tubing, and arterial filter, compartments 1 and 2, respectively, and allowed the observed volume data to be independently fitted. Photolytic conversion of DTIC to 2-AZA within the HLM was first order with rate constant, K_{35}, estimated from the in vitro perfusion data. Volumes for 2-AZA in the HLM and arterial filter (i.e., compartments 1 and 2) were the same as for DTIC. Measured concentration values for DTIC and 2-AZA in the HLM were estimated as the quotient of compartment 3/compartment 1 for DTIC and compartment 5/compartment 1 for 2-AZA. In order to maintain mass balance within the closed perfusion circuit and to account for the 25% of drug not recovered in the washout fluid, the in vitro model required modification. The arterial filter and HLM were added as potential binding surfaces, compartments 7–10, respectively for DTIC and 2-AZA. Linear and nonlinear binding to these surfaces was investigated. The results of the in vitro data fit, linear vs. nonlinear binding, are presented in Figure 3, and it appears that the data are best described by nonlinear binding since the decrease in levels following dosing was not adequately explained by linear binding and drug dilution because the volume of the system was considered constant (addition of the volume with the dose increased the overall volume by <7.5%). Discrepancies between the model predicted values and the experimental data (Fig. 3A,C) suggested a change in binding as a function of time (i.e., nonlinear binding) and photolytic degradation of DTIC in the filter, both being included in the in vitro model for DTIC and 2-AZA, resulting in improvement in the experimental data fitting (Fig. 3B,D). The HLM and filter binding function developed for the in vitro perfusion was used with some minor modification to describe the binding to these parts of the perfusion circuit for model development in the six subjects investigated.

Development of an In Vivo Model for Subject Perfusion

Development of the in vivo model required the inclusion of physiological parameters related to subject body size. The physiologic values for muscle volumes obtained from the literature [17] are presented in Table I. All values were based upon blood flow since DTIC has been shown to be ionized at physiologic pH and not to partition readily across membranes and is <15% protein bound [2]. Also, a tissue-to-plasma partition coefficient was not required to explain the DTIC plasma levels during perfusion since DTIC was administered throughout the entire perfusion period. The in vitro model (Fig. 2), had a compartment 11 added to the volume compartments 1 and 2 to account for volume changes within the muscle as a consequence of flow and pressure changes during extremity perfusion (Fig. 4). The initially measured 250 ml volume of the filter was assumed to remain constant with volume changes in the HLM being reflected by a concomitant change in the muscle volume in order to maintain relatively constant flows and pressures. In other words, only the total volume of the system was held constant. Observed HLM volumes for each subject were then fitted to determine the transfer rate constants between HLM, filter, and muscle using the measured changes in flow and volume within the HLM. In contrast to the in vitro model, which described distribution data for a closed system at equilibrium with constant flow and volume, the observed volumes and flows were not constant when the subject's perfused extremity was added to the circuit, although total volume was constant.

The amount of free DTIC within the muscle was represented by compartment 12. Measured venous concentrations of DTIC were calculated as the quotient of compartment 12/compartment 11. DTIC binding to muscle was represented by a reversible nonlinear binding, compartment 13 being connected to compartment 12. This compartment allows drug mass to absorb to muscle without a concommitant loss of volume from the system. Venous concentrations for 2-AZA were represented by the quotient of compartments 14/11. A similar equilibrium binding compartment was used for venous 2-AZA in muscle, which resulted in a poor fit of the arterial and venous data. An alternative scenario to describe the in vivo uptake of 2-AZA by the muscle is a delayed transfer to compartment 14, from a nonplasma pool, compartment 15, that releases drug slowly. Physiologically, this compartment may be within the muscles' extracellular space [18]. In effect, the compartment would provide a delay for the appearance of a portion of 2-AZA in plasma arriving via the nonplasma pool. In addition, the photolysis of DTIC within the filter was added resulting in a fraction of DTIC degraded within the filter being added directly to the 2-AZA plasma pool. This conversion is consistent with the structure of the perfusion system (Fig. 1).

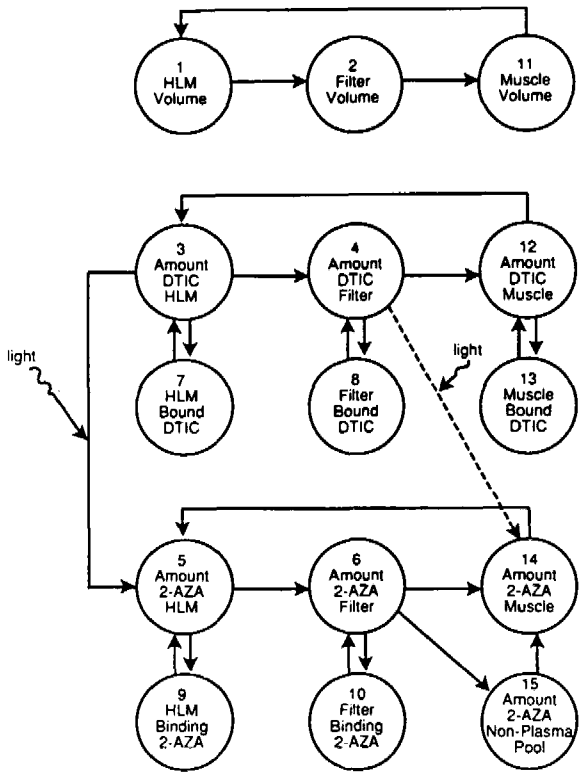

Fig. 4. In vivo model to describe the pharmacokinetics of DTIC and 2-AZA within the extracorporeal circuit containing the perfused extremity. The model contains nonlinear equilibrium binding for DTIC within the muscle (compartment 13), fractional transport of 2-AZA to a nonplasma pool (compartment 15) prior to appearance in plasma and photolytic conversion of DTIC to 2-AZA.

At the conclusion of the perfusion period, the fluid was removed from the circuit over 5 minutes. Correcting the dose for the amount of drug recovered, the drug "reversibly" bound to the filter, HLM, and within the patient at 5 minutes was estimated. When the in vivo model was developed, the parameters were adjusted individually for each subject using nonlinear least-squares techniques to obtain a best fit of the data, as judged both visually and by the sum of squared deviations of the model-calculated values from the observed data. Unknown parameters for drug binding were selected to minimize the weighted squared differences between model simulations and the experimental observations. Each term in the minimized function was inversely weighted by the assay variance of the data. All data were fitted simultaneously using the simulation analysis software (SAAM) program software [19]. Utilizing the final compartmental model and the subjects parameters, the recovery of the initial dose for each subject was estimated based upon HLM washout data and urinary recovery of DTIC, 2-AZA, and AIC. A theoretical estimate for recovery values as a function of washout volume/time and total washout time were predicted based upon the compartmental model in order to minimize the amount of drug re-

maining in the muscle to cause toxicity following the washout period.

Differential Equations

The following differential equations were used to describe the final model during limb perfusion:

1. dV-HLM/dt = −(Vo − HLM − Vfil) × Q-HLM/V-HLM
+Vmus × Q-HLM/Vmus + IC

2. dVfil/dt = V-HLM × Q-HLM/V-HLM − Vfil
× Q-HLM/Vfil

3. dVmus/dt = −Vmus × Q-HLM/Vmus + Vfil
× Q-HLM/Vfil

4. dDTIC-HLM/dt = − (f) DTIC-HLM
× Q-HLM/V-HLM
− (1-f) DTIC-HLM
× Q-HLM/V-HLM + DTICmus
× Q-HLM/Vmus
− (non-linear binding) + IC

5. dDTICfil/dt = (1-f) DTIC-HLM × Q-HLM/V-HLM
− DTICfil × Q-HLM/V-HLM (1-f′)
− DTICfil × Q-HLM/V-HLM (f′)
− (non-linear binding)

6. dDTICmus/dt = (1-f′) DTICfil × Q-HLM/Vfil
− DTICmus × QHLM/Vmus
−(nonlinear binding)

7. d2-AZA-HLM/dt = (f) DTIC-HLM
× Q-HLM/V-HLM + 2AZAmus
× Q-HLM/Vmus-2-AZA-HLM
× Q-HLM/V-HLM
− (non-linear binding)

8. d2-AZAfil/dt = 2-AZA-HLM × Q-HLM/V-HLM
− (f″)2-AZAfil
× Q-HLM/Vfil − (1-f″)2-AZAfil
× Q-HLM/Vfil
− (non-linear binding)

9. d2-AZAmus/dt = (1-f″) 2-AZAfil × Q-HLM/Vfil
+ (non linear input non plasma pool)
− 2-AZAmus × Q-HLM/Vmus
+ (f′) DTICfil × Q-HLM/Vfil

where:
V-HLM = (Vo-HLM-Vfil)
Vfil = volume of filter
Vmus = volume of muscle
f = fraction of DTIC converted to 2-AZA in HLM
f′ = fraction of DTIC converted to 2-AZA in filter
f″ = fraction of 2-AZA transported directly to venous blood
Q-HLM = flow in the HLM
DTIC HLM, fil, mus = amount of DTIC in HLM, filter, and muscle respectively
2-AZA HLM, fil, mus = amount of 2-AZA in HLM, filter, and muscle, respectively
Nonlinear binding—amount bound/unit time
IC—initial conditions.

Equations 1–9 can be expressed as concentration (i.e., dividing each by the appropriate volume term from equations 1–3). The nonlinear binding is considered to involve only the reversible removal of drug from the extracorporeal circuit without any concomitant removal of significant volume. The parameters obtained from the fit of this data were used to describe the binding of DTIC with subsequent 2-AZA formation and binding for the study subject data.

RESULTS

In Vitro Model

The experimental data for DTIC in the in vitro study indicated that the half-life for photolysis of DTIC to 2-AZA was ~105 minutes. Of note was the absence of AIC in the pump circuit. Recovery of drug was 75%, indicating that some drug was lost to the tubing and the oxygenator. Approximately 1% of the drug recovered from the in vitro study was 2-AZA. The fitted data for the perfusion circuit (Fig. 5) exhibits nonlinear binding of DTIC and 2-AZA to the HLM and filter as a function of time as described under pharmacokinetic methods. A similar binding pattern was used to described the in vitro binding (i.e., HLM and filter) when the patient was added to the extracorporeal perfusion circuit (Fig. 6A,B,C), although the binding for subjects was influenced by changes in volume (Fig. 6D).

In Vivo Model

Plasma levels of DTIC following perfusion of the isolated limb exhibited some accumulation in the arterial and venous plasma samples following the several doses of DTIC (Figs. 6 and 7). The peak levels were ~2–3 times those observed at the end of the perfusion in the arterial and venous samples. Arterial and venous levels of 2-AZA were ~40× less than the levels for DTIC within the perfusion circuit (Figs. 7 and 8). The fit of the data for patient 3 (Figs. 6 and 8) shows the nonlinearity in the binding rate constants over time. Arterial peak concentrations were 1.5 and 3 × venous levels for DTIC and 2-AZA, respectively.

In order to maintain physiologic conditions within the system, volume changes within the extremity were compensated by altering the flow in the HLM. These changes

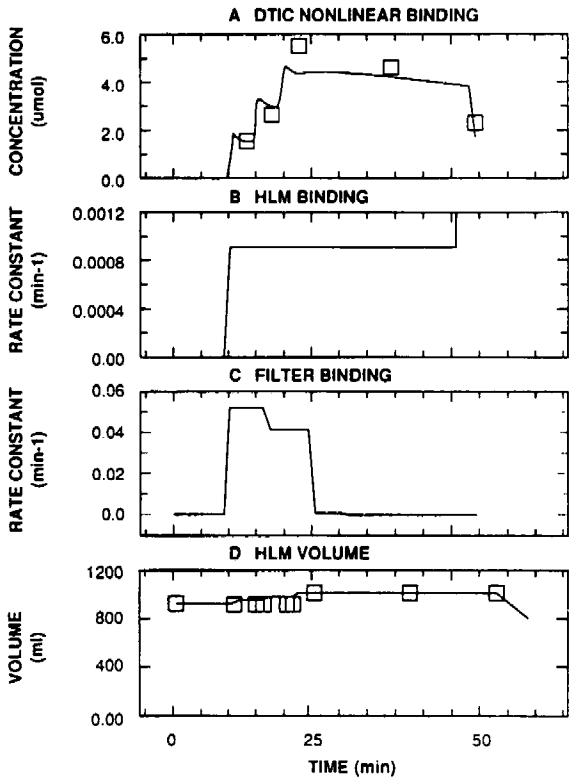

Fig. 5. Fitted data (**A**) using the in vitro model for the in vitro perfusion. The nonlinear binding of DTIC to the HLM and filter (**B,C**) and flow (**D**) within the HLM during the study are represented.

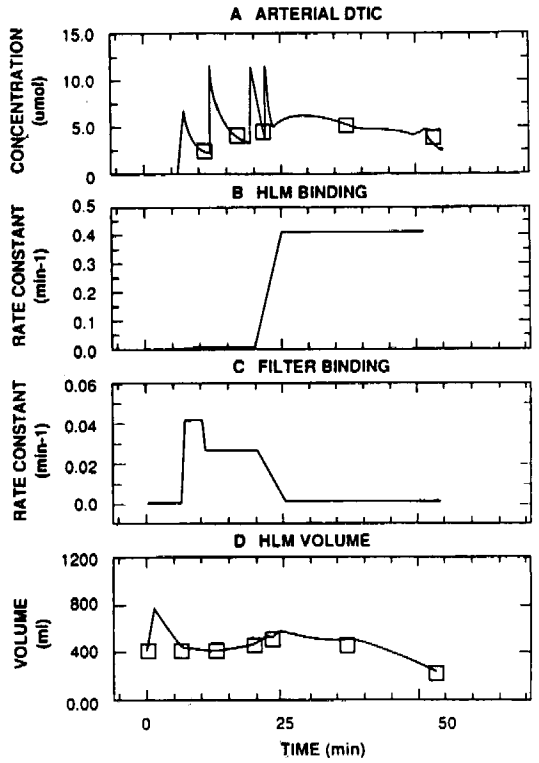

Fig. 6. Fitted DTIC arterial data (**A**) for subject 3 showing the changes in HLM and filter binding (**B,C**) as a function of time. The fitted changes in volume are also given in **D**.

in volume were fitted for each subject. A representative fit of the volume data for subject 3 is given in Figure 6D.

The systemic plasma levels for DTIC, 2-AZA (Table II) were roughly 100-fold less than the perfusion levels. Observed peripheral concentrations for AIC were $\sim 10\text{–}20 \times 10^{-3}$ μM (i.e., the same concentration range as systemic 2-AZA). Since many of the peripheral samples for all three compounds were either below the level of assay sensitivity for most subjects or were not taken in the recovery room, this data could not be readily modeled, which also made it impractical to develop a model for urinary excretion of the three compounds. Therefore, the in vivo model describes the perfusion kinetics. The drug appearance in the systemic circulation during perfusion was most likely related to the "leaking" of drug from the isolated limb. Although there appeared to be no pattern associated with these values, most subjects did show an increase when the tourniquet was removed at ~60 minutes into the study.

The amount of drug recovered in the washout and urine for the drug and its metabolites is given in Table III. Washout recovery ranged from 19–38% of the administered dose. Urinary recovery for the three subjects measured also showed a wide range of 2–22%. Combination of this data gave an overall average recovery of 32.6%. The percentage dose recovery values based upon observed data indicated that for most patients, more than one-half of the drug recovered originated from the washout procedure at the conclusion of the extremity perfusion (Table III). The recovery of the total dose of ^{99m}Tc HSA at the end of perfusion was in the range of 85–98% for patients. This indicated that >50% of the drug is retained in the extremity despite washout at the end of the perfusion. None of the patients developed tissue toxicity despite high levels of drug in the perfusion circuit. The patients were observed for compartmental syndrome, vascular thrombosis, and neurotoxicity.

A simulated recovery based upon the compartmental model for the data investigated the effect of an increased washout rate and/or time on the amount of DTIC and 2-AZA recovered in the washout fluid. The two scenarios had differing effects on recovery. When the rate of washout (ml/min) over the 5-minute time period was increased, recovery of drug decreased for all subjects (Fig. 9). However, when the washout time was increased from 5 minutes to 8 minutes, there was a substantial predicted increase in the amount of possible drug to be recovered in the washout fluid (Fig. 10).

DISCUSSION

The main purpose of regional chemotherapy is to achieve a high concentration of the drug in the affected

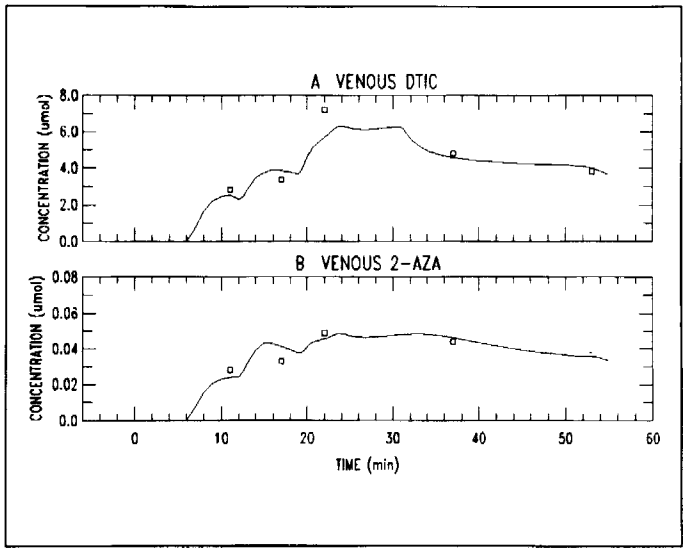

Fig. 7. Venous DTIC (A) and venous 2-AZA data for subject 3 fitted using the in vivo model.

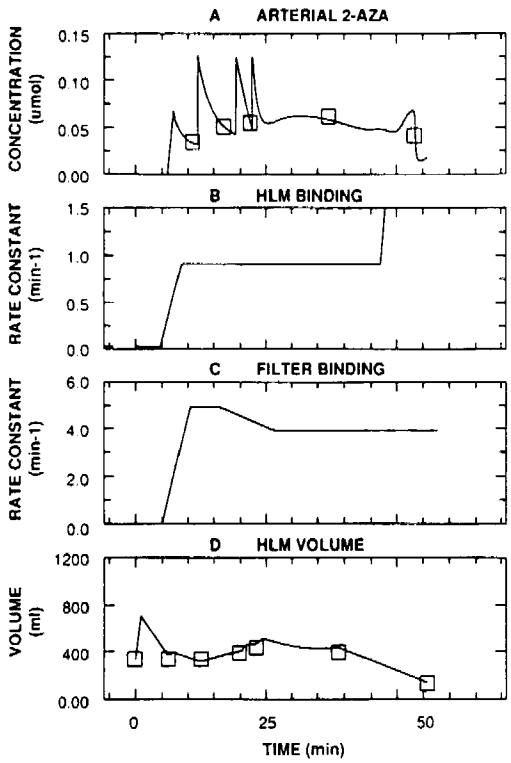

Fig. 8. 2-AZA fitted arterial plasma levels (**A**), HLM binding (**B**), and filter binding (**C**) as a function of time for subject 3. **D** gives the fitted observed changes in HLM volume over the period of the perfusion.

region without systemic exposure, thereby achieving the best therapeutic index while minimizing systemic toxic effects. The vascular isolation and perfusion of the affected extremity with larger doses of the chemotherapeutic agent achieve this ideal principle. However, even with minimal leakage of perfusate into the systemic circulation

TABLE II. Representative Systemic Plasma Concentration for DTIC 2-AZA and AIC During and After Extracorporeal Perfusion of Extremity for Subjects 3 and 5 Who Had Measurable Levels of DTIC and 2-AZA

Time (min) μmoles	DTIC[a] μmoles	2-AZA[b] μmoles	AIC[c]
		Subject 3	
50		0.01	—
65	0.12	0.02	—
80	0.13	0.02	—
		Subject 5	
22	0.04		0.018
37	0.04		0.019
42	0.05		0.019
67	0.05		—
375	0.05		0.020

[a] Dacarbazine.
[b] Azahypoxanthine.
[c] Carboxamide.

based upon DTIC levels, we noted hemotoxicity in 14 out of the first 40 patients perfused with high doses of DTIC [10]. Since prior studies did not include a complete description of the events within the perfusion circuit that would explain these observations, we undertook the study of DTIC pharmacokinetics during perfusion. Additionally, no prior study has reported the pharmacokinetics of DTIC perfusion in clinical situations. The peak level of DTIC in the perfusion circuit was quickly achieved following the last administered dose. This peak level is ~10-fold more than that achieved by systemic injection of DTIC in humans [6]. The dosage of DTIC used in the perfusion circuit was 100-fold greater than that used for systemic administration. The fitted model also predicted accumulation of DTIC in the venous perfusate (Fig. 7).

In contrast, the systemic levels of DTIC were 100-fold less because of the adequate vascular isolation of the

TABLE III. Percent of DTIC, AIC, and Degradation Product, 2-AZA, Recovered in Washout Fluid and Urine Compared to Percent of 99mTc Human Serum Albumin Dose Recovered in Washout Fluid for Patients Who Were Perfused with DTIC*

Patient	Washout	Urine	Total	^{99m}Tc HSA
1[a]	26.0	—	26.0	89
2[b]	19.8	2.7	22.5	85
3[b]	22.5	12.0	34.5	97
4[b]	20.9	22.4	43.3	86
5[b]	30.9	—	30.9	94
6[b]	38.9	—	38.9	98
*Mean ± SD	26.5 ± 7.3	12.36 ± 9.85	32.68 ± 7.82	91.5 ± 5.6

[a]Urine not collected in these subjects.
[b]Individual percent recovery in urine were:

Subject	3	4	2
DTIC (dacarbazine)	9.6	12.42	1.89
2-AZA (azahypoxanthine)	0.26	0.05	0.75
AIC (carboxamide)	2.1	9.96	0.06

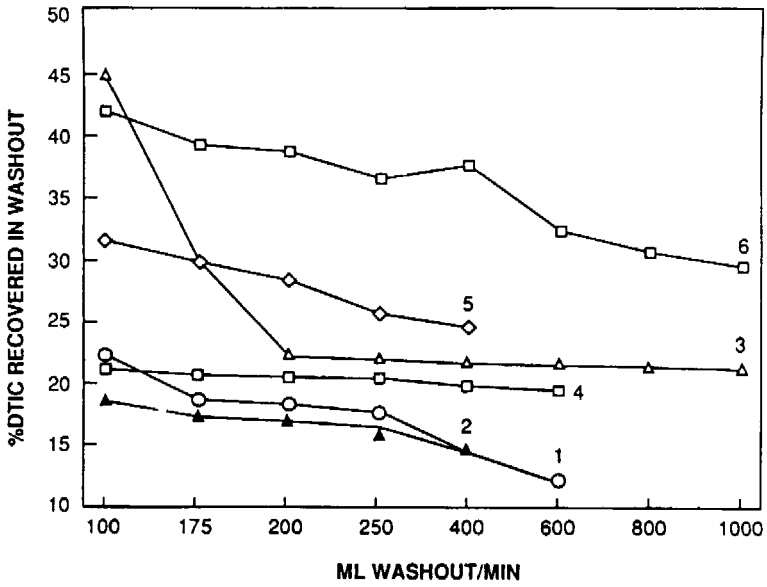

Fig. 9. Effect of increasing washout volume/time on the percentage of DTIC that would theoretically be recovered in the washout fluid, based upon the fitted data for each subject.

extemity during perfusion. This indicated that either a minimal amount of drug crossed over into the systemic circulation during perfusion or that the lower systemic levels resulted from the larger volume of the body and plasma for the drug to distribute. However, since intensive systemic sampling and modelling was not done for the peripheral data, some levels may have been higher than those observed.

We found that only a mean of 26% (range 19–38%) of the total drug was recovered in the washout solution. This raises two possibilities: (1) the drug is firmly bound by the tissues of the extremities (e.g., diffusion rate limited) and requires a longer period for washout, and (2) the firmly bound drug does not completely diffuse out of soft tissues by washout but is released slowly into the systemic circulation as the unchanged drug after the vasculature is reconnected. It should be noted that the compartmental model we developed to describe the data exhibited reversible binding, indicating that all drug should eventually be transported from the muscle. Therefore, the overall recovery of DTIC and its metabolites seen in Table III, although similar to that reported by Breithaupt [3], will not be quantitative.

An apparent clinical consequence of this result is to obtain better recovery of drug from the isolated extremity. A much longer washout time should be employed as indicated by the simulation in Figure 10.

It was noted that urinary excretion of unchanged DTIC over 24 hours ranged from 12% to 42% in patients. Similarly, AIC recovered in urine was 5–10% of the total

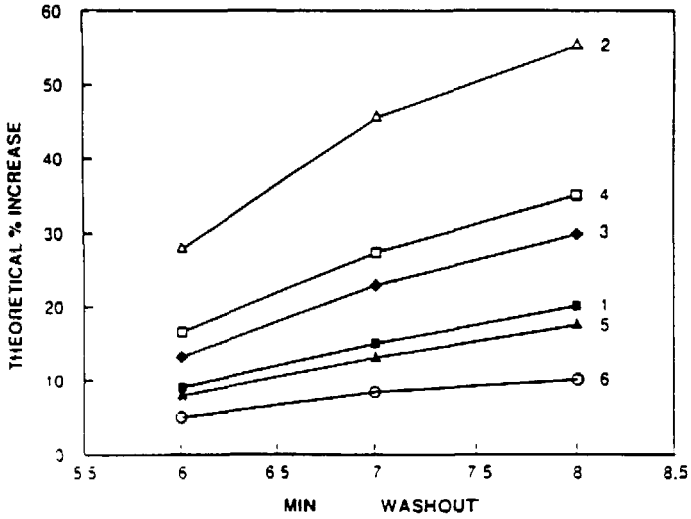

Fig. 10. Theoretical percent increase in the amount of DTIC recovered in the washout procedure by increasing the washout time while keeping the washout volume constant.

DTIC dose administered. The appearance of both DTIC and AIC in urine was noted after the perfusion and washout process.

The model developed to describe the pharmacokinetics of DTIC during isolated extremity perfusion provides a structure for organizing the observed data into a form suitable for testing theories related to DTIC. Although the kinetics for DTIC are complex, based upon the quality of the fits for the experimental data, this simple model provides a reasonable representation of the events occurring during extremity perfusion. The data also showed the appearance of small quantities of AIC in the systemic circulation. This is most likely to consequence of DTIC that leaked during perfusion or after the tourniquet was removed. This result is in contrast to other authors [3] who did not observe AIC in the systemic circulation. We infer that this conversion occurred mainly from the retained DTIC in tissues of the extremity that diffused into the systemic circulation after the washout and was metabolized to AIC by the liver.

Hyperthermia in the perfusion circuit raises the temperature of the perfusate to 40°C. This could affect the metabolism of DTIC. The degradation of DTIC solution in a hyperthermic bath without ultraviolet light was <2% in 1 hour, the time used for perfusion (Miles Laboratories, pers. comm.). We, therefore, do not think that the degradation of DTIC used with hyperthermia vitiated our observation.

CONCLUSIONS

Limb isolation perfusion achieves the intended goal of delivering large doses of DTIC to the extremity with minimal systemic exposure. The kinetics of DTIC are influenced by the nonspecific binding to the HLM and filters. Increased recovery of DTIC from the limb following perfusion can be realized by increasing the washout time.

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